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(71) Applicant: BAYLOR COLLEGE OF MEDICINE [US/US]; One Baylor Plaza, Houston, TX 77030-3498 (US).

(72) Inventors: BUTEL, Janet, S.; 3031 Albans Avenue, Houston, TX 77005 (US). LEE, Teh-Hsiu; 1922 Wyndale #3, Houston, TX 77030 (US). ELLEDGE, Stephen, J.; 2505 Wordsworth, Houston, TX 77030 (US).

(74) Agent: PAUL, Thomas, D.; Fulbright & Jaworski, Suite 5100, 1301 McKinney, Houston, TX 77010-3095 (US).

(54) Title: HEPATITIS B VIRUS INTERACTS WITH CELLULAR DNA REPAIR PROCESSES

(57) Abstract

A method of treating viral disease in an animal or human, comprising the step of interfering with the interaction of the viral protein with a DNA repair complex in the animal or human to be treated. Specific examples include inhibition of the interaction of the X protein from HBV and the XAP-1 protein of the DNA repair complex. The method can also be used for the treatment of cancer secondary to viral infection. Also, there is a nucleic acid sequence encoding the XAP-1 protein and the amino acid sequence of the XAP-1 protein. Antibodies to the protein can be used for diagnostic purposes and the XAP-1 gene can be used as the object of screening assays to detect genetic alterations.

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HEPATITIS B VIRUS INTERACTS WITH CELLULAR DNA REPAIR PROCESSES

This application is a Continuation-in-Part of United States Application Serial No. 08/133,932 filed October 11, 1993.

The present invention was made utilizing funds of the United States Government. The United States Government is entitled to certain rights under this invention.

Field of the Invention

This invention relates to methods of treating viral diseases, liver cancer secondary to viral diseases, diagnosing viral diseases and specific antibodies against antigens involved in interactions with proteins of viral origin. More particularly it relates to the nucleic acid sequence for XAP-1 and its amino acid sequence of the human XAP-1 protein, which is part of the DNA repair complex.

Background

Hepatitis B Virus (HBV): Clinical Importance

HBV is worldwide in distribution. It causes acute and chronic liver cell damage and hepatocellular carcinoma (HCC; liver cancer). HBV may cause long-term persistent infections, with the frequency depending on the age and immunologic status of the host at the time of infection, and genetic factors of both the virus and host. Persistent infections often result in serious liver disease, including cirrhosis and cancer. The prevalence rates of persistent infections range from as high as 20% in certain regions of China, Southeast Asia, and subsaharan Africa to less than 0.5% in North America. It is estimated there are approximately 300 million persistently infected carriers

of HBV worldwide. An estimated 1 million deaths annually are attributable to the harmful effects of HBV infection. At this time, no clinically useful antivirals against HBV are available.

HBV is transmitted in various ways, including from mother to offspring, by contact, and by parenteral and sexual routes. High-risk groups include parenteral drug abusers, institutionalized persons, health care personnel (surgeons, pathologists, and other physicians, dentists, nurses, laboratory technicians, and blood bank personnel), individuals who have recently received blood transfusions, hemodialysis patients and staff, highly promiscuous persons, and newborn infants born to mothers with hepatitis B. A variety of modes of HBV transmission and rates of persistent infections exist between endemic countries with high prevalence rates and nonendemic countries. In endemic areas, the major routes of infection are perinatal transmission from infected mothers to offspring and contact-associated transmission during the first years of life.

It is the chronic infection with HBV that is a major risk factor of HCC. Viral infection usually persists for several decades before the emergence of HCC, and the risk of tumor development increases with the duration of chronic infection. Chronic carriers are about 200 times more likely to develop HCC than uninfected persons living in the same area. Nearly 50% of Chinese males with chronic HBV infections will eventually suffer HCC. Analyses of liver tissue from patients with chronic HBV infection have identified both replicative intermediates (indicative of active viral replication) and integrated forms of HBV DNA.

HBV Genome and Gene Products

HBV is a member of the hepadnavirus family, a group of hepatotropic, DNA-containing viruses. Additional animal hepadnaviruses include the woodchuck hepatitis virus (WHV), ground squirrel hepatitis virus, and a virus of tree squirrels. Avian hepadnaviruses include the duck hepatitis B virus (DHBV) and DHBV-related viruses from geese, grey herons, and other species of ducks. Although minor differences exist, hepadnaviruses are similar in morphology, genome structure, and pathogenesis. All hepadnaviruses are characterized by a restrictive host and tissue tropism and are associated with

both acute and chronic liver disease. Infectious HBV is present mainly in the blood of infected patients, but it is also found in other body fluids, such as saliva, urine, and semen. The liver is the target organ, but blood cells may also be infected.

The HBV genome consists of partially double-stranded DNA, approximately 3,200 base pairs (bp) in length; it represents the smallest genome of any virus known to infect man. DNA sequence analyses have demonstrated 90—98% nucleotide sequence homology among different HBV isolates, and the genome structures of the HBV isolates share many features in common with other hepadnaviruses. The full-length (i.e., 3,200 bp) DNA strand of the HBV genome is of minus polarity and is complementary to all HBV mRNAs. In contrast, the positive strand is complementary at its 5' end to the first 224 bp of the negative strand, but has a variable 3' end (ranging from 53 to 88% of unit length).

The organization of the HBV genome is remarkably efficient. Analysis of different HBV isolates has revealed the conservation of four long open reading frames (ORFs) within the genome which encode specific viral proteins, including the virus nucleocapsid (core antigen or HBcAg), envelope glycoproteins (surface antigen or HBsAg), polymerase (product of the P gene), and a protein from the X gene.

HBV Replication

Replication of HBV begins with the attachment of the virus particle to susceptible cells, such as hepatocytes. Following entry into hepatocytes, the core component is released from the virion, and the partially double-stranded DNA genome is converted to a covalently closed circular (CCC) form that can be detected in the cell nucleus. The CCC DNA then serves as template for the production of HBV mRNAs. The 3.5-kb pre-genome transcript is encapsidated, via a packaging signal located near the 5' end of the RNA, into newly synthesized core particles where it serves as template for the HBV reverse transcriptase. An RNase-H-like activity within the HBV polymerase removes the RNA template, as the negative-strand DNA is synthesized. Viral replicative intermediates, consisting of full-length minus-strand DNA plus variable-length (20—40%) positive-strand DNA, are encapsidated within the

core particle during normal virus replication. Virus replication is completed as the DNA-containing core particles bud from the cell surface.

The integration of HBV DNA into chromosomal DNA, although not a part of normal viral replication, occurs during chronic infection with HBV. Integrated HBV can be detected in most but not all HCCs that arise in HBV-positive individuals and is usually present in 1—4 genome copies per cell. The percentage of liver tumors that are HBV positive approaches 95% in high-HBV-endemic areas such as China, and viral DNA may be detected in approximately 80—85% of HBV-related HCCs from other regions of the world. The analysis of many HBV inserts cloned from HCCs has established that a preferred site for recombination of viral DNA with cellular DNA is located near the DR region of the viral genome. As replicative intermediates share structural features with many integrated HBV forms (i.e., one end near the DR region, with variable lengths of the positive-strand DNA), it is possible that replicative intermediates may serve as the template for integration of viral DNA.

Integration of HBV DNA is not required for viral replication, so the viral DNA insertions detected in the chromosomal DNA of HCCs most probably occur via illegitimate recombination. Such recombinational events are thought to occur at random locations. Gross chromosomal alterations are frequently observed at the site of viral integration and include deletions, duplications, and translocations. The effect of these chromosomal alterations on the hepatocyte will depend on the identity of genes nearby the insertion site and the extent to which gene expression is altered. Although HBV integration occurs at random within chromosomes, there is a subsequent nonrandom selection for cells containing viral integration events in specific chromosomes during the evolution of a tumor. Of 28 HBV inserts cloned from HCCs and mapped to human chromosomes, 61% have been assigned to chromosomes 3, 11, 17, and 18. When considering the mutagenic consequence of HBV integration, the data suggest that the disruption of genes on chromosomes 3, 11, 17, and 18 may be particularly important in the genesis of subsets of HCCs.

HBV X Protein

The X ORF represents the smallest ORF of the HBV genome and has the potential to encode a protein 146 to 154 amino acids in length, depending on the isolate of HBV. The X ORF overlaps with parts of the P and pre-C ORFs and encompasses several regulatory regions of the genome, including both DRs, enhancer II, and the C gene promoter. The X ORF peptide sequence is highly conserved among different viral isolates. Although detection of the X protein within HBV-infected cells remains difficult, the presence of anti-X protein antibodies in the sera of HBV-infected patients provides evidence that the X ORF is expressed during natural infection. Antibodies to X are detected most often in patients with chronic hepatitis, liver cirrhosis, and/or hepatoma.

DNA transfection approaches using the cloned X gene have clearly demonstrated that the X gene product can transactivate a wide variety of viral and cellular promoters. The heterogeneity of the elements responsive to X suggests that the X gene product exerts its effect by a mechanism other than direct binding to a specific DNA sequence, probably functioning via effects on cellular factors. The ability of the X gene to transactivate the expression of HBV RNA suggests an important regulatory role for X during viral replication. Indeed, animal studies have indicated the requirement for X expression for virus growth *in vivo*.

Specific functions attributable to X protein have been difficult to pinpoint. In addition to being a transactivator, X protein has been reported to be a serine protease inhibitor, and to activate the cellular protein kinase C signalling pathway. It has been suggested that the X protein may affect other cellular processes, besides transcription, that are regulated by cellular kinases.

Cellular DNA Repair Mechanisms

Accidental lesions occur continually in the DNA of eucaryotic cells. DNA damaging agents include thermal fluctuations, ultraviolet (UV) irradiation, environmental toxins, and man-made chemicals. It is estimated that thousands of DNA nucleotides are damaged in a cell every day by such chemical processes. If unattended, this would quickly lead to unacceptably

high rates of mutations in germ cells (which would affect maintenance of the species) and in somatic cells (which would adversely affect the individual). Uncontrolled cell proliferation (i.e., cancer) is one deleterious outcome of the accumulation of genetic changes in a cell.

Cells have a complex system for recognizing and repairing damage in the DNA, in order to maintain the fidelity of the genome. This process is called DNA repair. Based on yeast genetic studies, it is estimated that eucaryotic cells contain more than 50 genes involved in DNA repair functions, reflecting the great importance of this process to the cell.

Individuals with the autosomal recessive genetic disease called xeroderma pigmentosum (XP) have a defect in the DNA repair system. Such individuals are sun-sensitive (displaying an abnormal sensitivity to UV radiation) and have a marked predisposition toward skin cancer (2000-fold increased frequency). Somatic cell fusion experiments using cells derived from XP patients have defined seven complementation groups (A—G), suggesting that a multienzyme complex is involved in efficient DNA repair. XP patients are defective in the "bulky lesion" repair mechanism that is believed to be responsible for scanning and repairing large changes in the structure of the DNA double helix.

Two groups have described a protein that binds to damaged DNA that may represent the defective protein in XP group E patients (Takao *et al.*, *Nucleic Acid Res.* **21**:4111-4118, 1993; Hwang and Chu, *Biochemistry* **32**:1657-1666, 1993). The monkey gene "UV-DDB" was cloned by Takao *et al.*; the human cognate of the UV-DDB gene was mapped to chromosome 11. Hwang and Chu purified the protein (XPE-binding factor) from human placenta. Both proteins are about the same size (125—127 kDa) and have a high binding affinity for damaged DNA. Both groups conclude their isolate is probably involved in the recognition step of the excision repair pathway. However, as the proteins differ in some biochemical properties, it is not yet clear if the two groups have recovered the same protein.

A number of yeast excision repair genes (RAD) and rodent DNA-repair genes (ERCC) have been isolated. It has recently been shown that the RAD2, ERCC5, and XPGC genes are equivalent. This emphasizes the striking homology between eukaryotic nucleotide-excision repair genes.

Of interest is the recent observation that repair protein XPBC/ERCC3 is part of the basal transcription factor TFIIH. This suggests that the same cellular proteins are involved in two different aspects of DNA metabolism: initiation of transcription and excision repair.

Summary of the Invention

An object of the present invention is a method for diagnosing viral infection.

An object of the present invention is a method for diagnosing cancer secondary to viral infection.

A further object of the present invention is the provision of a nucleic acid sequence for XAP-1.

Another aspect of the present invention is the provision of an amino acid sequence for the XAP-1 protein.

Another object of the present invention is a method for monitoring viral infection or cancer secondary to viral infection.

Another aspect of the present invention is the use of the XAP-1 gene as the object of screening assays to detect genetic alterations indicating a possible elevated risk of developing cancer.

Thus, in accomplishing the foregoing objects, there is provided in accordance with one aspect of the present invention a method of treating viral diseases in an animal or human, comprising the step of interfering with the interaction of a viral protein with a DNA repair complex in the animal or human to be tested. This same procedure can be used also to treat liver cancer secondary to viral infection. Other aspects of the invention include a nucleic acid sequence coding for the XAP-1 protein as well as the amino acid sequence for the XAP-1 protein and antibodies which bind to the XAP-1 protein or bind to the separate peptides of the XAP-1 protein.

The antibodies can be used to detect the infection, detect the cancer, monitor the effectiveness of therapy or monitor the stages of the cancer or infection.

Other objects, features and advantages will be apparent and more readily understood from reading the following specification and by references to the accompanying drawings forming a part thereof. Examples of the

presently preferred embodiments of the invention are given for the purpose of disclosure.

Brief Description of the Drawings

Figure 1 shows the transcriptional activation by reconstitution of GAL4 activity. (A) Transcription of *lac*Z (detected using chromogenic substrate X-GAL) does not occur when GAL4 does not bind GAL4 upstream activator sequence (UAS_G). (B) GAL4, which contains a DNA-binding domain (BIND) and a transcription activation domain (ACT), binds to UAS_G which results in activation of transcription. Bait (C) or prey (D) plasmids alone do not induce transcription. (E) Interaction of bait protein X and prey protein Y results in transcriptional activity.

Figure 2 shows the nucleic acid sequence which encodes the human XAP-1 protein.

Figure 3 shows the amino acid sequence of the XAP-1 protein.

Figure 4 shows the plasmid precursor to the bait plasmid pAS-1-X. The GAL4 DNA-binding domain (amino acids Nos. 1-147) are fused in frame with X ORF. The Trp gene is used as a selective marker.

Figure 5 shows the prey plasmid pACT. The GAL4 activation domain (amino acid Nos. 768-881) is fused with a cDNA library made from EBV-transformed human lymphocytes. The Leu gene is used as a selective marker. The host cell is the yeast strain Y153 and the *Lac*Z gene and His3 gene are controlled by GAL4 responsive elements.

Figure 6 shows the immunoprecipitation of labeled yeast extracts with anti-X antiserum. Lane one is the negative control. Lane two is the immunoprecipitate from yeast cells co-expressing X + LBP (Laminin-binding protein). Lane three is the immunoprecipitate from yeast cells co-expressing X and XAP-1. Anti-X antibody co-precipitated a 140 KD protein.

Figure 7 shows the hydrophilicity plot for XAP-1. Underlined regions 1 and 2 refer to peptides 1 and 2 used to generate antipeptide antibodies in rabbits.

Figure 8 shows that XAP-1 synthesized *in vitro* in rabbit reticulocyte lysates primed with XAP-1 mRNA is recognized by anti-peptide antisera in immunoprecipitation tests. Lane 1 is a sample of the radiolabeled lysate;

XAP-1 migrates at about 127 kD. Lane 2 is a sample of lysate reacted with control serum. Lanes 3 and 4 are samples of lysate reacted with anti-peptide 2 serum and anti-peptide 1 serum, respectively.

Figure 9 shows the expression of XAP-1 in HEpG2 cells (human heptoma-derived cells). Panel A is a Northern blot showing that XAP-1 mRNA is 4.4 kb in size. XAP-1 protein is expressed in the HEpG2 cells (Panels B and C). Cells were metabolically labeled with [^{35}S]methionine, extracted, and immunoprecipitated with anti-peptide 2 antiserum (Panel B). XAP-1 (127 kD) plus several associated cellular proteins were recovered. When unlabeled HEpG2 cells were extracted, reacted with anti-peptide serum, and then analyzed in an immunoblot reaction using anti-peptide serum, XAP-1 protein was detected (Panel C).

Figure 10 shows the association between X and XAP-1 when X is expressed as a GST fusion protein. Lane 1 is radiolabeled *in vitro* translation of XAP-1. The translation mixtures containing labeled XAP-1 were applied to gluthathione-sepharose beads containing immobilized control GST protein (lane 2) or GST-X fusion protein (lane 3). Bound protein was eluted and analyzed by SDS-PAGE. Note that XAP-1 was recovered using the GST-X fusion protein.

Figure 11 shows the procedure used in the experiment in Figure 10 for verifying the interaction between X and XAP-1.

Detailed Description

It will be readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

As used herein, DNA repair function refers to the general steps of DNA repair, including: (1) recognition of a lesion in the DNA; (2) incision of the damaged strand on both sides of the lesion; (3) excision of the damaged nucleotides; (4) synthesis of new DNA by copying the "good" complementary strand as template; and (5) ligation (sealing) of the nick left in the repaired strand. The end result of DNA repair is the restoration of two good copies of the DNA sequence.

As used herein, DNA repair complex refers to the chemical components involved in the intricate system of recognizing and repairing damage to DNA, particularly the multienzyme complexes that have been found to be associated with the recognition of a lesion in DNA, incision and removal of damaged nucleotides, synthesis of new DNA and ligation of the nick in the repaired strand.

Gene therapy, as used herein, refers generally to the method of treating an individual with genetic material to treat a disease or other pathophysiological condition.

As used herein, cancer secondary to HBV infection refers to the occurrence of carcinomas and other cancers in an individual as the result of infection with Hepatitis B Virus.

One aspect of the present invention is a method of treating viral disease in a non-human animal or a human, comprising the step of interfering with the interaction of a viral protein with a DNA repair complex in the animal or human to be treated. One specific example would be the treatment of Hepatitis B Virus (HBV) viral disease in non-human animals and humans. In this procedure, the interaction of the X protein of HBV with the XAP-1 protein of the DNA repair complex is inhibited. Thus, acute and chronic infections may be treated and possibly cured by the use of inhibitory substances to block the interaction of X protein with the cellular DNA repair machinery. Such an inhibitory substance and approaches can include, but are not limited to:

(a) Antiviral drugs to reduce expression of X protein.

(b) A decoy synthetic peptide mimicking the interactive domain of either X or XAP-1 (or related proteins) to block their interaction.

(c) Gene therapy vectors encoding such blocking peptides.

(d) Antisense constructs or inhibitory oligonucleotides directed against HBV sequences that would block the synthesis of X protein.

(e) Intracellular antibodies able to block the interaction of X with XAP-1 or other components of the repair complex.

(f) Chemicals to enhance the level of nuclease activity or other activities of the repair process so that the repair pathway would not be overwhelmed by HBV DNA and X protein.

(g) Substances that would prevent the migration of HBV cores to the intracellular location where they contact the DNA repair mechanism.

One skilled in the art readily recognizes that these approaches can be used to inhibit the interaction of a viral protein with the DNA repair complex.

Another alternative embodiment includes the method of treating cancer secondary to viral infection in an animal or human, comprising the steps of interfering with the interaction of a viral protein with a DNA repair complex in the animal or human to be treated. One skilled in the art readily understands that in treating the cancer, one can reduce, delay or prevent the effects of viral protein on the cellular DNA repair system. This would allow the repair process to monitor and repair chromosomal DNA damage, preventing the accumulations of mutations in the cell. In one specific example of the present invention, liver cancer secondary to HBV infection can be treated by inhibiting the interaction of the X protein of HBV with the XAP-1 protein of DNA repair complex.

Another embodiment is the use of the XAP-1 gene in diagnostic tests to identify those at increased risk of developing cancer. If the XAP-1 gene were to be mutated spontaneously or by exposure to viral infection or environmental factors, the loss of the normal DNA repair function might predispose cells to carcinogenic changes. Genetic errors would rapidly accumulate in those cells, some of which would affect important growth-regulatory genes. Similarly, individuals with inherited mutations in the XAP-1 gene might be cancer-prone. Diagnostic tests based on the detection of altered forms of the XAP-1 gene or gene product would be useful to identify those at increased risk of cancer development. At-risk individuals could then be monitored to detect cancers at their earliest stage, when treatment is most successful.

Although the specific examples above for treatment of disease and for treatment of cancer involve the HBV, other viral infections that induce cellular DNA damage or that involve the DNA repair system at some stage in the virus host interaction may be prevented, suppressed, modulated and/or cured using an inhibitory substance to block the interaction of specific viral proteins with the DNA repair mechanisms of the host cell, and similar to that described for HBV and HBV X protein. This can include members of the

adeno-, arena-, bunya-, calici-, corona-, flavi-, hepadna-, herpes-, ortho-, papova-, paramyxo-, parvo-, picorna-, pox-, reo-, retro-, rhabdo-, and togavirus families, viroids, and agents not yet classified, and specifically would include hepatitis C virus. It would include target tissues in any organ system in the body, including but not limited to liver, gastrointestinal tract, respiratory system, skin, blood and blood-forming tissues, and the brain and nervous system.

Another approach is the use of gene therapy. These approaches include blocking HBV X function and its interactions with cellular DNA repair components. One skilled in the art also readily recognizes that gene therapy approaches can be directed to XAP-1, related proteins, and other components of the multienzyme DNA repair complex to modulate its ability to correct DNA damage induced by toxic chemicals, environmental toxins, viruses or other infectious agents, pharmaceuticals used to treat other conditions, or inherited genetic defects including mutator phenotypes.

Another embodiment of the present invention is the XAP-1 gene nucleic acid sequence which encodes for the XAP-1 protein, shown in Figure 2.

Another embodiment of the present invention is the amino acid sequence of the XAP-1 protein which is shown in Figure 3.

Polyclonal or monoclonal antibodies can be made against two synthetic peptides derived from the sequences of XAP-1, as well as to the whole XAP-1 protein or other fragments of the XAP-1. This procedure includes preparing the polyclonal or monoclonal antibodies against the synthetic peptides from the sequence, against authentic intact protein purified from eucaryotic cells or protein expressed using various express systems, including not only modified or unmodified intact protein, but the fragments of the protein also. Examples of these antibodies are shown in the examples of antibodies prepared against peptide 1 and peptide 2.

In a specific embodiment of the present invention, the antibodies are used for diagnostic purposes. These antibodies can be used to identify defects in xeroderma pigmentosum patients, stage HBV infections or liver cancer development and to monitor the effectiveness of various therapeutic treatments using immunohistochemical, immunofluorescence,

immunoprecipitation, immunoblot, enzyme-linked immunosorbent assays, or other immunological methods.

Example 1

Identification of HBV X Protein Interactive Cellular Proteins Using the Yeast Two-Hybrid System

Background about system: Understanding the functions of the HBV X protein requires a knowledge of the protein-protein interactions that occur in different target cells and at different stages in the viral life cycle. The difficulty encountered so far in detecting X protein synthesized in HBV-infected human cells or in transgenic mouse cells has precluded studies of cellular proteins able to complex with the viral polypeptide. Even when found, X may well be an unstable, low-abundance protein which will further complicate studies of protein-protein interactions. The present invention utilizes the yeast two-hybrid system to circumvent these problems.

The two-hybrid system was described by Fields and Song, *Nature* 340:245-246, 1989, and modified by Elledge, [Durfee et al. *Genes and Dev.* 7:555-569, 1993]. The procedure is a genetic method to identify and clone genes that interact with a protein of interest using *in vivo* complementation in yeast. The system relies on the properties of the yeast GAL4 protein, which contains two separable domains responsible for transcriptional activation and DNA binding. Plasmids encoding two hybrid proteins, one consisting of the GAL4 DNA-binding domain fused to protein of interest X (bait) and the other consisting of the GAL4 transcription activator domain fused to test protein Y (prey), are introduced into yeast. Interaction between bait and prey proteins leads to transcriptional activation of a reporter gene containing a binding site for GAL4. In principle, a mammalian cDNA library (prey plasmids) can be quickly screened provided neither participant (bait or prey) is self-activating. A schematic depicting transcription activation using the two-hybrid system, with *lacZ* as reporter gene, is shown in Figure 1. The two-hybrid system has several advantages over other commonly used methods to study protein-protein interactions: It is more sensitive than immunoprecipitation; purified proteins are not required; and it can be used to isolate genes encoding proteins that are normally expressed at a low level.

The bait component of the two-hybrid system: The cloning vector was pAS1, which carries as a selective marker TRP1 (Figure 4). A bait plasmid that expressed GAL4 DNA-binding domain—X fusion protein was constructed using the HBV X gene. *Saccharomyces cerevisiae* host strain Y153 was used. Transformed cells were plated on synthetic complete (SC) media lacking tryptophan. As stable propagation of bait plasmids in yeast does not guarantee expression of the cloned test gene, HBV X protein expression was verified by immunoprecipitation of ^{35}S-labeled proteins from sonically lysed yeast cells, using our X antisera.

Bait proteins that self-activate reporter genes cannot be used. The suitability of the X bait plasmids for the two-hybrid system was tested using a *lacZ* reporter assay and a 3-aminothiazole (3-AT) susceptibility assay. The *lacZ* reporter assay checks for activation of the yeast -galactosidase reporter gene. Transformed yeast cells were bound to a reinforced nitrocellulose filter, permeabilized in liquid nitrogen, and then incubated overnight in buffer containing X-gal; the colonies turn blue if *lacZ* is activated. The X plasmid did not display self-activation.

The prey component of the two-hybrid system: To search for interactive proteins, an activation-domain tagged cDNA expression library was co-transformed into yeast expressing the HBV X bait plasmid. Prey plasmids were derived from a lambda-phage (λ) cDNA expression vector (λ-ACT) (Figure 5). λ-ACT encodes a plasmid that automatically excises, recircularizes, and propagates when grown in the proper *E. coli* strain, obviating the need for subcloning λ-amplified cDNAs. The cDNA library used was derived from Epstein-Barr virus-immortalized human lymphocytes.

Recipient yeast containing bait plasmids were transformed with library DNA using a lithium sorbitol transformation protocol. Library transformed cells were placed for 3 hr in liquid SC media lacking His, Trp, and Leu to establish transformants and activate HIS3 transcription and then plated onto solid selective media. Colonies that grew after 3—5 days were tested using the *lacZ* reporter assay. Blue colonies were kept for further analysis.

Screens to rule out false positives: Some blue colonies able to grow on selective media may not be true positives due to specific bait and prey protein interactions. Approaches to rule out false positives included the nonspecific

interactor test. In this test, false-positives were ruled out by determining if they reacted nonspecifically with a panel of indicators expressed as bait fusion proteins. The panel contained lamin c, human cyclin D, truncated p53, and yeast protein SNF1. Prey fusion proteins that interacted with some or all panel proteins were considered to be false positives. One true positive that presumably encoded an X-associated protein was designated XAP-1 (X-associated protein 1). The laminin-binding protein and the beta subunit of G protein are partially X-specific.

Identification of HBV X interactive proteins: True positive prey plasmids were DNA sequenced and an identity scan performed using a databank search. Although the XAP-1 gene was not represented on the first screen, it later appeared in GenBank (accession no. L20216) as a related sequence for a UV-damaged DNA-binding (UV-DDB) protein recovered from a monkey cell cDNA library. The monkey UV-DDB gene is about 98% homologous to the human XAP-1 gene at both the nucleotide and amino acid levels. (Fifty-one nucleotide mismatches and one amino acid mismatch.)

Preparation of immunological reagents against XAP-1: We selected hydrophilic sequences from XAP-1, based on analysis of the gene sequence, and prepared anti-peptide antisera to be used for subsequent protein characterization (Figure 7). The peptides used were the following: peptide 1 - REKEFNKGPWKQENVE (amino acids 198—213); peptide 2 - QYDDGSGMKREATA (amino acids 1113—1126). Antibodies were raised that recognized XAP-1 synthesized *in vitro* in rabbit reticulocyte lysates primed with XAP-1 mRNA, as well as endogenous protein synthesized in human hepatoma-derived HEpG2 cells (Figures 8 and 9).

Proof of protein-protein interaction: Two approaches were used to verify HBV X protein interaction with XAP-1. Yeast cells expressing the two proteins were labeled with [^{35}S]methionine, extracted, and the lysate precipitated with anti-X antibody; GAL4—XAP-1 fusion protein was co-immunoprecipitated (Figure 6). In a second approach to prove binding, X was expressed as a glutathione S-transferase (GST) fusion protein in *E. coli*. The GST-fusion protein was immobilized on glutathione-sepharose beads, and ^{35}S-labeled *in vitro* translation mixtures containing the XAP-1 protein were applied to the column. Bound protein was eluted and analyzed by

SDS-PAGE. XAP-1 was recovered (Figure 10). An outline of the procedure is shown in Figure 11.

Example 2

Model of Significance to HBV Infections And Disease Pathogenesis

The model is that HBV usurps a normal DNA repair pathway in the cell to convert its partially double-stranded DNA genome to a covalently closed circular form, a necessary step in the virus life cycle. This step must occur with each progeny DNA molecule that is generated, either in newly infected cells, or as new rounds of replication are initiated in persistently infected cells. A cellular process presumably accomplishes this step; the viral polymerase functions to reverse transcribe pregenomic RNA to a partial double-stranded DNA, and inhibition of viral polymerase activity reportedly had no effect on generation of CCC DNA. Primer removal, and plus-strand completion must be early steps in the initiation of new rounds of replication. The steps necessary to convert the incomplete HBV genome to an intact infectious form are reminiscent of the repair process designed to correct damaged cellular DNA.

It is believed that the HBV X protein is required for the recruitment and/or functioning of the cellular repair proteins to repair the HBV genome or to trigger a series of changes that makes the hepatocyte more permissive for viral replication. The involvement of the X protein is to direct the cellular complex from a cellular site to HBV DNA, to stabilize the interaction of the multienzyme complex with HBV DNA, to displace or inhibit one or more enzymatic processes that are part of the normal DNA repair process that might be deleterious to HBV DNA, to enhance the activity of the complex to make it more beneficial for HBV, and/or to alter the function of the repair process in some other way.

Therefore, if the involvement of the cellular DNA repair mechanism in the repair and completion of the HBV genome is blocked, the replication of HBV is inhibited. It is thus possible to eliminate chronic infections with HBV, as well as to treat acute infections.

This model helps explain the role of HBV in liver cancer. The DNA repair proteins are in close proximity to chromosomal DNA and, as the genomic HBV DNA is being repaired, the viral DNA is, on occasion, accidentally integrated into the chromosome. If an important cellular gene were interrupted or affected, this could have profound effects on the cell. Inhibiting this on-going repair process of HBV DNA would decrease the number of integration events of HBV DNA and reduce the likelihood of eventual development of HCC. In addition, the involvement of X protein in redirecting the DNA repair process may be efficient enough that it interferes with normal repair of chromosomal DNA, resulting in the accumulation of mutations and genetic instability, eventually leading to cancer development. Therefore, blocking the interaction of X with the cellular repair mechanism will decrease the development of HCC.

This model explains several other poorly understood observations in the HBV system. The proposed function of X as a protease inhibitor might serve to dysregulate the cellular DNA repair complex by inhibiting a specific cleavage of a member of the complex in order to accentuate a function especially beneficial to HBV repair. As it now appears that some of the same cellular proteins are involved in initiation of transcription, excision repair, and DNA replication, this could explain the function of X protein as a promiscuous transcriptional activator. The X protein may be altering to a greater or lesser extent components of the transcription complex at the same time that it is modifying the DNA repair mechanism to meet the needs for HBV DNA repair. These effects on cellular processes would occur in cells expressing X protein from an integrated HBV genome, even in the absence of replicating HBV, because of the ability of X protein to interact with the cellular target proteins. The X protein has never been observed to exhibit DNA-binding activity. It is probable that X protein interaction with XAP-1 and possibly other members of the repair complex is necessary to enable X to affect the DNA-based processes.

Although the involvement of the X protein is required for the recruitment of the repair complex to repair HBV DNA, it is not unexpected that X has been reported to be dispensable for virus replication in tissue culture. Established cells in culture frequently contain mutations and display

altered patterns of gene expression; redundant biochemical pathways are expressed that would be normally repressed in hepatocytes *in vivo*. There are many examples of viral genes that are essential for viral growth in a host organism that are not necessary for viral growth in tissue culture cells.

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods, procedures and techniques described herein, as well as the antibodies, nucleic acid sequence and amino acid sequences are presently representative of the preferred embodiments, are intended to be exemplary, and are not intended as limitations on the scope. Changes therein and other uses which are encompassed within the spirit of the invention or defined by the scope of the appended claims will occur to those skilled in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Butel, Janet S.
Lee, Teh-Hsiu
Elledge, Stephen J.

(ii) TITLE OF INVENTION: Hepatitis B Virus Interacts With Cellular DNA Repair Processes

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fulbright & Jaworski
(B) STREET: 1301 McKinney, Suite 5100
(C) CITY: Houston
(D) STATE: Texas
(E) COUNTRY: U.S.A.
(F) ZIP: 77010-3095

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/238,396
(B) FILING DATE: 05-May-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Paul, Thomas D.
(B) REGISTRATION NUMBER: 32,714
(C) REFERENCE/DOCKET NUMBER: D-5628-C1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 713/651-5151
(B) TELEFAX: 713/651-5246
(C) TELEX: 762829

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3423 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGTCGTACA ACTACGTGGT AACGGCCCAG AAGCCCACCG CCGTGAACGG CTGCGTGACC 60

GGACACTTTA CTTCGGCCGA AGACTTAAAC CTGTTGATTG CCAAAAACAC GAGATTAGAG 120

ATCTATGTGG TCACCGCCGA GGGGCTTCGG CCTGTCAAAG AGGTGGGCAT GTATGGGAAG 180

ATTGCGGTCA TGGAGCTTTT CAGGCCCAAG GGGGAGAGCA AGGACCTGCT GTTTATCTTG 240

ACAGCGAAGT ACAATGCCTG CATCCTGGAG TATAAACAGA GTGGCGAGAG CATTGACATC 300

ATTACGCGAG CCCATGGCAA TGTCCAGGAC CGCATTGGCC GCCCCTCAGA GACCGGCATT 360

ATTGGCATCA TTGACCCTGA GTGCCGGATG ATTGGCCTGC GTCTCTATGA TGGCCTTTTC 420

AAGGTTATTC CACTAGATCG CGATAATAAA GAACTCAAGG CCTTCAACAT CCGCCTGGAG 480

GAGCTGCATG TCATTGATGT CAAGTTCCTA TATGGTTGCC AAGCACCTAC TATTTGCTTT 540

GTCTACCAGG ACCCTCAGGG GCGGCACGTA AAAACCTATG AGGTGTCTCT CCGAGAAAAG 600

GAATTCAATA AGGGCCCTTG GAAACAGGAA AATGTCGAAG CTGAAGCTTC CATGGTGATC 660

GCAGTCCCAG AGCCCTTTGG GGGGGCCATC ATCATTGGAC AGGAGTCAAT CACCTATCAC 720

AATGGTGACA AATACCTGGC TATTGCCCCT CCTATCATCA AGCAAAGCAC GATTGTGTGC 780

CACAATCGAG TGGACCCTAA TGGCTCAAGA TACCTGCTGG GAGACATGGA AGGCCGGCTC 840

TTCATGCTGC TTTTGGAGAA GGAGGAACAG ATGGATGGCA CCGTCACTCT CAAGGATCTC 900

CGTGTAGAAC TCCTTGGAGA GACCTCTATT GCTGAGTGCT TGACATACCT TGATAATGGT 960

GTTGTGTTTG TCGGGTCTCG CCTGGGTGAC TCCCAGCTTG TGAAGCTCAA CGTTGACAGT 1020

AATGAACAAG GCTCCTATGT AGTGGCCATG GAAACCTTTA CCAACTTAGG ACCCATTGTC 1080

GATATGTGCG TGGTGGACCT GGAGAGGCAG GGGCAGGGGC AGCTGGTCAC TTGCTCTGGG 1140

GCTTTCAAGG AAGGTTCTTT GCGGATCATC CGGAATGGAA TTGGAATCCA CGAGCATGCC 1200

AGCATTGACT TACCAGGCAT CAAAGGATTA TGGCCACTGC GGTCTGACCC TAATCGTGAG 1260

ACTGATGACA CTTTGGTGCT CTCTTTTGTG GGCCAGACAA GAGTTCTCAT GTTAAATGGA 1320

GAGGAGGTAG AAGAAACCGA ACTGATGGGT TTCGTGGATG ATCAGCAGAC TTTCTTCTGT 1380

GGCAACGTGG CTCATCAGCA GCTTATCCAG ATCACTTCAG CATCGGTGAG GTTGGTCTCT 1440

CAAGAACCCA AAGCTCTGGT CAGTGAATGG AAGGAGCCTC AGGCCAAGAA CATCAGTGTG 1500

GCCTCCTGCA ATAGCAGCCA GGTGGTGGTG GCTGTAGGCA GGGCCCTCTA CTATCTGCAG 1560

ATCCATCCTC AGGAGCTCCG GCAGATCAGC CACACAGAGA TGGAACATGA AGTGGCTTGC 1620

TTGGACATCA CCCCATTAGG AGACAGCAAT GGACTGTCCC CTCTTTGTGC CATTGGCCTC 1680

TGGACGGACA TCTCGGCTCG TATCTTGAAG TTGCCCTCTT TTGAACTACT GCACAAGGAG 1740

ATGCTGGGTG GAGAGATCAT TCCTCGCTCC ATCCTGATGA CCACCTTTGA GAGTAGCCAT 1800

TACCTCCTTT GTGCCTTGGG AGATGGAGCG CTTTTCTACT TTGGGCTCAA CATTGAGACA 1860

GGTCTGTTGA GCGACCGTAA GAAGGTGACT TTGGGCACCC AGCCCACCGT ATTGAGGACT 1920

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AGCAGCAACC ACAAATTGGT CTTCTCAAAT GTCAACCTCA AGGAAGTGAA CTACATGTGT 2040

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CTGTCCAGCA GTGTAAGCTC CAGCAAGCTG TTCTCCAGCA GCACTGCTCC TCATGAGACC 2340

TCCTTTGGAG AAGAGGTGGA GGTGCACAAC CTACTTATCA TTGACCAACA CACCTTTGAA 2400

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GTGGCTGAAA AGGAAGTGAA AGGGGCCGTG TACTCTATGG TGGAATTTAA CGGGAAGCTG 2640

TTAGCCAGCA TCAATAGCAC GGTGCGGCTC TATGAGTGGA CAACAGAGAA GGAGCTGCGC 2700

ACTGAGTGCA ACCACTACAA CAACATCATG GCCCTCTACC TGAAGACCAA GGGCGACTTC 2760

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AACTTTGAAG AGATTGCTCG AGACTTTAAT CCCAACTGGA TGAGTGCTGT GGAAATCTTG 2880

GATGATGACA ATTTTCTGGG GGCTGAAAAT GCCTTTAACT TGTTTGTGTG TCAAAAGGAT 2940

AGCGCTGCCA CCACTGACGA GGAGCGGCAG CACCTCCAGG AGGTTGGTCT TTTCCACCTG 3000

GGCGAGTTTG TCAATGTCTT TTGCCACGGC TCTCTGGTAA TGCAGAATCT GGGTGAGACT 3060

TCCACCCCCA CACAAGGCTC GGTGCTCTTC GGCACGGTCA ACGGCATGAT AGGGCTGGTG 3120

ACCTCACTGT CAGAGAGCTG GTACAACCTC CTGCTGGACA TGCAGAATCG ACTCAATAAA 3180

GTCATCAAAA GTGTGGGGAA GATCGAGCAC TCCTTCTGGA GATCCTTTCA CACCGAGCGG 3240

AAGACAGAAC CAGCCACAGG TTTCATCGAC GGTGACTTGA TTGAGAGTTT CCTGGATATT 3300

AGCCGCCCCA AGATGCAGGA GGTGGTGGCA AACCTACAGT ATGACGATGG CAGCGGTATG 3360

AAGCGAGAGG CCACTGCAGA CGACCTCATC AAGGTTGTGG AGGAGCTAAC TCGGATCCAT 3420

TAG 3423

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Tyr Asn Tyr Val Val Thr Ala Gln Lys Pro Thr Ala Val Asn
1 5 10 15

Gly Cys Val Thr Gly His Phe Thr Ser Ala Glu Asp Leu Asn Leu Leu
20 25 30

Ile Ala Lys Asn Thr Arg Leu Glu Ile Tyr Val Val Thr Ala Glu Gly
35 40 45

Leu Arg Pro Val Lys Glu Val Gly Met Tyr Gly Lys Ile Ala Val Met
50 55 60

Glu Leu Phe Arg Pro Lys Gly Glu Ser Lys Asp Leu Leu Phe Ile Leu
65 70 75 80

Thr Ala Lys Tyr Asn Ala Cys Ile Leu Glu Tyr Lys Gln Ser Gly Glu
85 90 95

Ser Ile Asp Ile Ile Thr Arg Ala His Gly Asn Val Gln Asp Arg Ile
100 105 110

Gly Arg Pro Ser Glu Thr Gly Ile Ile Gly Ile Ile Asp Pro Glu Cys
115 120 125

Arg Met Ile Gly Leu Arg Leu Tyr Asp Gly Leu Phe Lys Val Ile Pro
130 135 140

Leu Asp Arg Asp Asn Lys Glu Leu Lys Ala Phe Asn Ile Arg Leu Glu
145 150 155 160

Glu Leu His Val Ile Asp Val Lys Phe Leu Tyr Gly Cys Gln Ala Pro
165 170 175

Thr Ile Cys Phe Val Tyr Gln Asp Pro Gln Gly Arg His Val Lys Thr
180 185 190

Tyr Glu Val Ser Leu Arg Glu Lys Glu Phe Asn Lys Gly Pro Trp Lys
195 200 205

Gln Glu Asn Val Glu Ala Glu Ala Ser Met Val Ile Ala Val Pro Glu
210 215 220

Pro Phe Gly Gly Ala Ile Ile Ile Gly Gln Glu Ser Ile Thr Tyr His
225 230 235 240

Asn Gly Asp Lys Tyr Leu Ala Ile Ala Pro Pro Ile Ile Lys Gln Ser
245 250 255

Thr Ile Val Cys His Asn Arg Val Asp Pro Asn Gly Ser Arg Tyr Leu
260 265 270

Leu Gly Asp Met Glu Gly Arg Leu Phe Met Leu Leu Leu Glu Lys Glu
275 280 285

Glu Gln Met Asp Gly Thr Val Thr Leu Lys Asp Leu Arg Val Glu Leu
290 295 300

Leu Gly Glu Thr Ser Ile Ala Glu Cys Leu Thr Tyr Leu Asp Asn Gly
305 310 315 320

Val Val Phe Val Gly Ser Arg Leu Gly Asp Ser Gln Leu Val Lys Leu
325 330 335

Asn Val Asp Ser Asn Glu Gln Gly Ser Tyr Val Val Ala Met Glu Thr
340 345 350

Phe Thr Asn Leu Gly Pro Ile Val Asp Met Cys Val Val Asp Leu Glu
355 360 365

Arg Gln Gly Gln Gly Gln Leu Val Thr Cys Ser Gly Ala Phe Lys Glu
370 375 380

Gly Ser Leu Arg Ile Ile Arg Asn Gly Ile Gly Ile His Glu His Ala
385 390 395 400

Ser Ile Asp Leu Pro Gly Ile Lys Gly Leu Trp Pro Leu Arg Ser Asp
405 410 415

Pro Asn Arg Glu Thr Asp Asp Thr Leu Val Leu Ser Phe Val Gly Gln
420 425 430

Thr Arg Val Leu Met Leu Asn Gly Glu Glu Val Glu Glu Thr Glu Leu
435 440 445

Met Gly Phe Val Asp Asp Gln Gln Thr Phe Phe Cys Gly Asn Val Ala
450 455 460

His Gln Gln Leu Ile Gln Ile Thr Ser Ala Ser Val Arg Leu Val Ser
465 470 475 480

Gln Glu Pro Lys Ala Leu Val Ser Glu Trp Lys Glu Pro Gln Ala Lys
485 490 495

Asn Ile Ser Val Ala Ser Cys Asn Ser Ser Gln Val Val Val Ala Val
500 505 510

Gly Arg Ala Leu Tyr Tyr Leu Gln Ile His Pro Gln Glu Leu Arg Gln
515 520 525

Ile Ser His Thr Glu Met Glu His Glu Val Ala Cys Leu Asp Ile Thr
530 535 540

Pro Leu Gly Asp Ser Asn Gly Leu Ser Pro Leu Cys Ala Ile Gly Leu
545 550 555 560

Trp Thr Asp Ile Ser Ala Arg Ile Leu Lys Leu Pro Ser Phe Glu Leu
565 570 575

Leu His Lys Glu Met Leu Gly Gly Glu Ile Ile Pro Arg Ser Ile Leu
580 585 590

Met Thr Thr Phe Glu Ser Ser His Tyr Leu Leu Cys Ala Leu Gly Asp
595 600 605

Gly Ala Leu Phe Tyr Phe Gly Leu Asn Ile Glu Thr Gly Leu Leu Ser
610 615 620

Asp Arg Lys Lys Val Thr Leu Gly Thr Gln Pro Thr Val Leu Arg Thr
625 630 635 640

Phe Arg Ser Leu Ser Thr Thr Asn Val Phe Ala Cys Ser Asp Arg Pro
645 650 655

Thr Val Ile Tyr Ser Ser Asn His Lys Leu Val Phe Ser Asn Val Asn
660 665 670

Leu Lys Glu Val Asn Tyr Met Cys Pro Leu Asn Ser Asp Gly Tyr Pro
675 680 685

Asp Ser Leu Ala Leu Ala Asn Asn Ser Thr Leu Thr Ile Gly Thr Ile
690 695 700

Asp Glu Ile Gln Lys Leu His Ile Arg Thr Val Pro Leu Tyr Glu Ser
705 710 715 720

Pro Arg Lys Ile Cys Tyr Gln Glu Val Ser Gln Cys Phe Gly Val Leu
725 730 735

Ser Ser Arg Ile Glu Val Gln Asp Thr Ser Gly Gly Thr Thr Ala Leu
740 745 750

Arg Pro Ser Ala Ser Thr Gln Ala Leu Ser Ser Ser Val Ser Ser Ser
755 760 765

Lys Leu Phe Ser Ser Ser Thr Ala Pro His Glu Thr Ser Phe Gly Glu
770 775 780

Glu Val Glu Val His Asn Leu Leu Ile Ile Asp Gln His Thr Phe Glu
785 790 795 800

Val Leu His Ala His Gln Phe Leu Gln Asn Glu Tyr Ala Leu Ser Leu
805 810 815

Val Ser Cys Lys Leu Gly Lys Asp Pro Asn Thr Tyr Phe Ile Val Gly
820 825 830

Thr Ala Met Val Tyr Pro Glu Glu Ala Glu Pro Lys Gln Gly Arg Ile
835 840 845

Val Val Phe Gln Tyr Ser Asp Gly Lys Leu Gln Thr Val Ala Glu Lys
850 855 860

Glu Val Lys Gly Ala Val Tyr Ser Met Val Glu Phe Asn Gly Lys Leu
865 870 875 880

Leu Ala Ser Ile Asn Ser Thr Val Arg Leu Tyr Glu Trp Thr Thr Glu
885 890 895

Lys Glu Leu Arg Thr Glu Cys Asn His Tyr Asn Asn Ile Met Ala Leu
900 905 910

Tyr Leu Lys Thr Lys Gly Asp Phe Ile Leu Val Gly Asp Leu Met Arg
915 920 925

Ser Val Leu Leu Leu Ala Tyr Lys Pro Met Glu Gly Asn Phe Glu Glu
930 935 940

Ile Ala Arg Asp Phe Asn Pro Asn Trp Met Ser Ala Val Glu Ile Leu
945 950 955 960

Asp Asp Asp Asn Phe Leu Gly Ala Glu Asn Ala Phe Asn Leu Phe Val
965 970 975

Cys Gln Lys Asp Ser Ala Ala Thr Thr Asp Glu Glu Arg Gln His Leu
980 985 990

Gln Glu Val Gly Leu Phe His Leu Gly Glu Phe Val Asn Val Phe Cys
995 1000 1005

His Gly Ser Leu Val Met Gln Asn Leu Gly Glu Thr Ser Thr Pro Thr
1010 1015 1020

Gln Gly Ser Val Leu Phe Gly Thr Val Asn Gly Met Ile Gly Leu Val
1025 1030 1035 1040

Thr Ser Leu Ser Glu Ser Trp Tyr Asn Leu Leu Leu Asp Met Gln Asn
1045 1050 1055

Arg Leu Asn Lys Val Ile Lys Ser Val Gly Lys Ile Glu His Ser Phe
1060 1065 1070

Trp Arg Ser Phe His Thr Glu Arg Lys Thr Glu Pro Ala Thr Gly Phe
1075 1080 1085

Ile Asp Gly Asp Leu Ile Glu Ser Phe Leu Asp Ile Ser Arg Pro Lys
1090 1095 1100

Met Gln Glu Val Val Ala Asn Leu Gln Tyr Asp Asp Gly Ser Gly Met
1105 1110 1115 1120

Lys Arg Glu Ala Thr Ala Asp Asp Leu Ile Lys Val Val Glu Glu Leu
1125 1130 1135

Thr Arg Ile His
1140

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating viral disease in an animal or human, comprising the step of interfering with the interaction of a viral protein with a DNA repair complex in the animal or human to be treated.

2. The method of claim 1, wherein the viral disease is caused by the Hepatitis B Virus (HBV) and the interaction of the X protein of HBV with XAP-1 of the DNA repair complex is inhibited.

3. A method of treating cancer secondary to viral infection in an animal or human, comprising the steps of interfering with the interaction of a viral protein with a DNA repair complex in the animal or human to be treated.

4. The method of claim 3, wherein the liver cancer is secondary to HBV infection and the interaction of the X protein of HBV with XAP-1 of the DNA repair complex is inhibited.

5. A method of identifying persons at increased risk of developing cancer, comprising detecting alterations in the XAP-1 gene of an individual.

6. The method of claim 5, wherein said XAP-1 gene alterations are detected by one or more of the following techniques: restriction enzyme analysis, polymerase chain reaction assays, nucleic acid hybridization, and/or using any of various immunological assays.

7. The nucleic acid sequence of XAP-1 protein shown in Figure 2.

8. The amino acid sequence of XAP-1 protein shown in Figure 3.

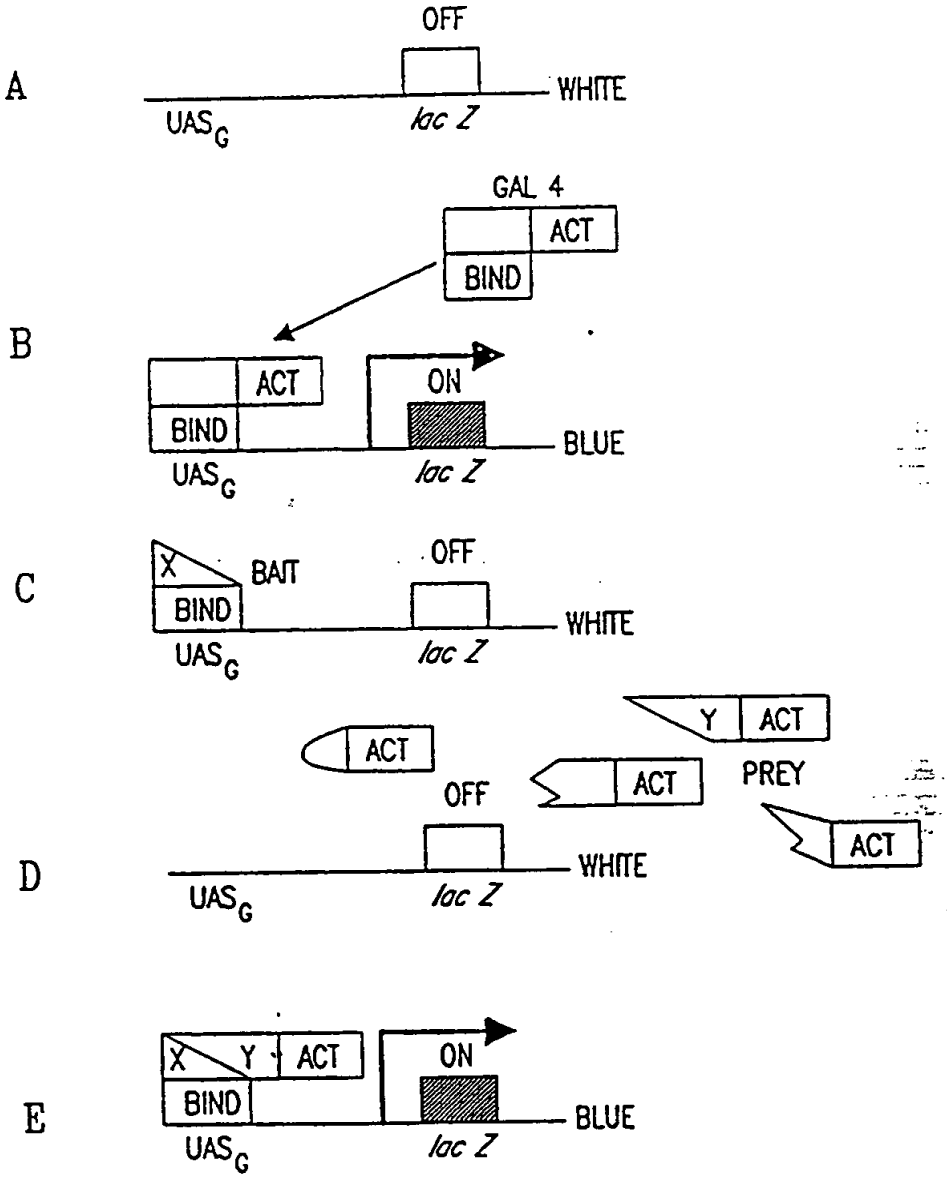
9. An antibody which binds to the protein of claim 6.

10. The antibody of claim 7 which binds to peptide 1, or peptide 2 or both.

11. A method of detecting patients with HBV infection, comprising the step of combining a sample from said patient with the antibody of claim 7 and measuring the amount of antibody-antigen interaction.

12. A method of monitoring the effectiveness of therapeutic treatments, comprising the step of combining a sample from said patient with the antibody of claim 9 and measuring the amount of antibody-antigen interaction.

13. A method of determining the stage of liver cancer secondary to HBV infection, comprising the step of combining a sample from said patient with the antibody of claim 9 and measuring the amount of antibody-antigen interaction.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BIND
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loc Z
C
X
BAIT
BIND
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E
X
Y
ACT
BIND
ON
BLUE
UAS$_G$
loc Z

Figure 1 A-E

FIGURE 2 (1/2)

ATGTCGTACAACTACGTGGTAACGGCCCAGAAGCCCACCGCCGTGAACGGCT
GCGTGACCGGACACTTTACTTCGGCCGAAGACTTAAACCTGTTGATTGCCAAA
AACACGAGATTAGAGATCTATGTGGTCACCGCCGAGGGGCTTCGGCCTGTCA
AAGAGGTGGGCATGTATGGGAAGATTGCGGTCATGGAGCTTTTCAGGCCCAA
GGGGGAGAGCAAGGACCTGCTGTTTATCTTGACAGCGAAGTACAATGCCTGC
ATCCTGGAGTATAAACAGAGTGGCGAGAGCATTGACATCATTACGCGAGCCC
ATGGCAATGTCCAGGACCGCATTGGCCGCCCCTCAGAGACCGGCATTATTGG
CATCATTGACCCTGAGTGCCGGATGATTGGCCTGCGTCTCTATGATGGCCTTT
TCAAGGTTATTCCACTAGATCGCGATAATAAAGAACTCAAGGCCTTCAACAT
CCGCCTGGAGGAGCTGCATGTCATTGATGTCAAGTTCCTATATGGTTGCCAAG
CACCTACTATTTGCTTTGTCTACCAGGACCCTCAGGGGCGGCACGTAAAAACC
TATGAGGTGTCTCTCCGAGAAAAGGAATTCAATAAGGGCCCTTGGAAACAGG
AAAATGTCGAAGCTGAAGCTTCCATGGTGATCGCAGTCCCAGAGCCCTTTGG
GGGGGCCATCATCATTGGACAGGAGTCAATCACCTATCACAATGGTGACAAA
TACCTGGCTATTGCCCCTCCTATCATCAAGCAAAGCACGATTGTGTGCCACAA
TCGAGTGGACCCTAATGGCTCAAGATACCTGCTGGGAGACATGGAAGGCCGG
CTCTTCATGCTGCTTTTGGAGAAGGAGGAACAGATGGATGGCACCGTCACTCT
CAAGGATCTCCGTGTAGAACTCCTTGGAGAGACCTCTATTGCTGAGTGCTTGA
CATACCTTGATAATGGTGTTGTGTTTGTCGGGTCTCGCCTGGGTGACTCCCAG
CTTGTGAAGCTCAACGTTGACAGTAATGAACAAGGCTCCTATGTAGTGGCCA
TGGAAACCTTTACCAACTTAGGACCCATTGTCGATATGTGCGTGGTGGACCTG
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GTTCTTTGCGGATCATCCGGAATGGAATTGGAATCCACGAGCATGCCAGCATT
GACTTACCAGGCATCAAAGGATTATGGCCACTGCGGTCTGACCCTAATCGTG
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TTAAATGGAGAGGAGGTAGAAGAAACCGAACTGATGGGTTTCGTGGATGATC
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GGTGGTGGTGGCTGTAGGCAGGGCCCTCTACTATCTGCAGATCCATCCTCAGG
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TCTGGACGGACATCTCGGCTCGTATCTTGAAGTTGCCCTCTTTTGAACTACTG
CACAAGGAGATGCTGGGTGGAGAGATCATTCCTCGCTCCATCCTGATGACCA
CCTTTGAGAGTAGCCATTACCTCCTTTGTGCCTTGGGAGATGGAGCGCTTTTC
TACTTTGGGCTCAACATTGAGACAGGTCTGTTGAGCGACCGTAAGAAGGTGA
CTTTGGGCACCCAGCCCACCGTATTGAGGACTTTTCGTTCTCTTTCTACCACCA
ACGTCTTTGCTTGTTCTGACCGCCCCACTGTCATCTATAGCAGCAACCACAAA
TTGGTCTTCTCAAATGTCAACCTCAAGGAAGTGAACTACATGTGTCCCCTCAA
TTCAGATGGCTATCCTGACAGCCTGGCGCTGGCCAACAATAGCACCCTCACC
ATTGGCACCATCGATGAGATCCAGAAGCTGCACATTCGCACAGTTCCCCTCTA
TGAGTCTCCAAGGAAGATCTGCTACCAGGAAGTGTCCCAGTGTTTCGGGGTC
CTCTCCAGCCGCATTGAAGTCCAAGACACGAGTGGGGGCACGACAGCCTTGA
GGCCCAGCGCTAGCACCCAGGCTCTGTCCAGCAGTGTAAGCTCCAGCAAGCT
GTTCTCCAGCAGCACTGCTCCTCATGAGACCTCCTTTGGAGAAGAGGTGGAG
GTGCACAACCTACTTATCATTGACCAACACACCTTTGAAGTGCTTCATGCCCA

FIGURE 2 (2/2)

CCAGTTTCTGCAGAATGAATATGCCCTCAGTCTGGTTTCCTGCAAGCTGGGCA
AAGACCCCAACACTTACTTCATTGTGGGCACAGCAATGGTGTATCCTGAAGA
GGCAGAGCCCAAGCAGGGTCGCATTGTGGTCTTTCAGTATTCGGATGGAAAA
CTACAGACTGTGGCTGAAAAGGAAGTGAAAGGGGCCGTGTACTCTATGGTGG
AATTTAACGGGAAGCTGTTAGCCAGCATCAATAGCACGGTGCGGCTCTATGA
GTGGACAACAGAGAAGGAGCTGCGCACTGAGTGCAACCACTACAACAACAT
CATGGCCCTCTACCTGAAGACCAAGGGCGACTTCATCCTGGTGGGCGACCTT
ATGCGCTCAGTGCTGCTGCTTGCCTACAAGCCCATGGAAGGAAACTTTGAAG
AGATTGCTCGAGACTTTAATCCCAACTGGATGAGTGCTGTGGAAATCTTGGAT
GATGACAATTTTCTGGGGGCTGAAAATGCCTTTAACTTGTTTGTGTGTCAAAA
GGATAGCGCTGCCACCACTGACGAGGAGCGGCAGCACCTCCAGGAGGTTGGT
CTTTTCCACCTGGGCGAGTTTGTCAATGTCTTTTGCCACGGCTCTCTGGTAATG
CAGAATCTGGGTGAGACTTCCACCCCCACACAAGGCTCGGTGCTCTTCGGCA
CGGTCAACGGCATGATAGGGCTGGTGACCTCACTGTCAGAGAGCTGGTACAA
CCTCCTGCTGGACATGCAGAATCGACTCAATAAAGTCATCAAAAGTGTGGGG
AAGATCGAGCACTCCTTCTGGAGATCCTTTCACACCGAGCGGAAGACAGAAC
CAGCCACAGGTTTCATCGACGGTGACTTGATTGAGAGTTTCCTGGATATTAGC
CGCCCCAAGATGCAGGAGGTGGTGGCAAACCTACAGTATGACGATGGCAGCG
GTATGAAGCGAGAGGCCACTGCAGACGACCTCATCAAGGTTGTGGAGGAGCT
AACTCGGATCCATTAG

FIGURE 3

MSYNYVVTAQKPTAVNGCVTGHFTSAEDLNLLIAKNTRLEIYVVTAEGLRPVKE
VGMYGKIAVMELFRPKGESKDLLFILTAKYNACILEYKQSGESIDIITRAHGNVQD
RIGRPSETGIIGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFNIRLEELHVIDVK
FLYGCQAPTICFVYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAV
PEPFGGAIIIGQESITYHNGDKYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGR
LFMLLLEKEEQMDGTVTLKDLRVELLGETSIAECLTYLDNGVVFVGSRLGDSQL
VKLNVDSNEQGSYVVAMETFTNLGPIVDMCVVDLERQGQGQLVTCSGAFKEGS
LRIIRNGIGIHEHASIDLPGIKGLWPLRSDPNRETDDTLVLSFVGQTRVLMLNGEE
VEETELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVSQEPKALVSEWKEPQAK
NISVASCNSSQVVVAVGRALYYLQIHPQELRQISHTEMEHEVACLDITPLGDSNG
LSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSILMTTFESSHYLLCALGDG
ALFYFGLNIETGLLSDRKKVTLGTQPTVLRTFRSLSTTNVFACSDRPTVIYSSNHK
LVFSNVNLKEVNYMCPLNSDGYPDSLALANNSTLTIGTIDEIQKLHIRTVPLYESP
RKICYQEVSQCFGVLSSRIEVQDTSGGTTALRPSASTQALSSSVSSSKLFSSSTAPH
ETSFGEEVEVHNLLIIDQHTFEVLHAHQFLQNEYALSLVSCKLGKDPNTYFIVGTA
MVYPEEAEPKQGRIVVFQYSDGKLQTVAEKEVKGAVYSMVEFNGKLLASINSTV
RLYEWTTEKELRTECNHYNNIMALYLKTKGDFILVGDLMRSVLLLAYKPMEGNF
EEIARDFNPNWMSAVEILDDDNFLGAENAFNLFVCQKDSAATTDEERQHLQEVG
LFHLGEFVNVFCHGSLVMQNLGETSTPTQGSVLFGTVNGMIGLVTSLSESWYNL
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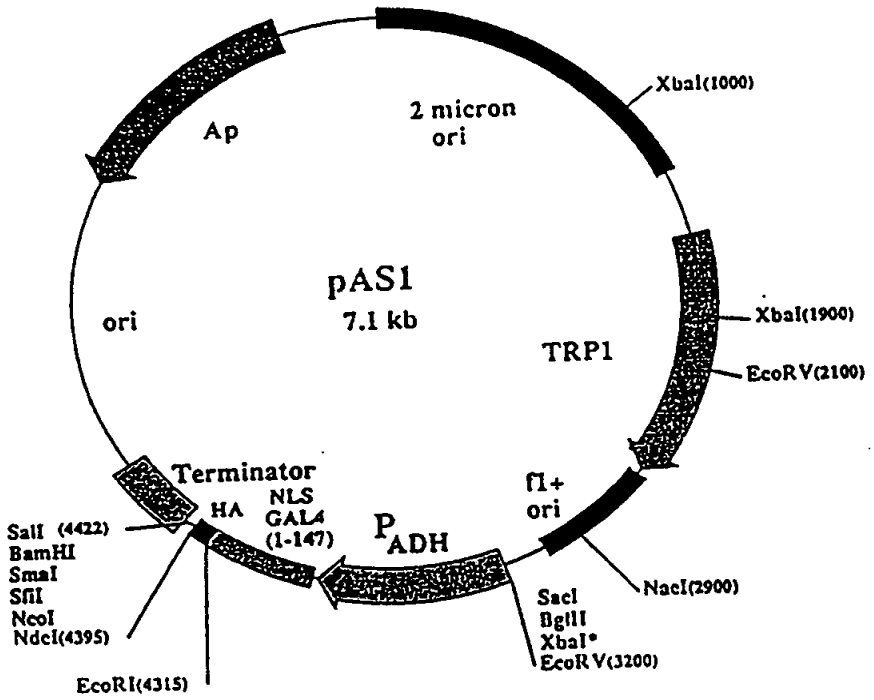
EVVANLQYDDGSGMKREATADDLIKVVEELTRIH


Ap
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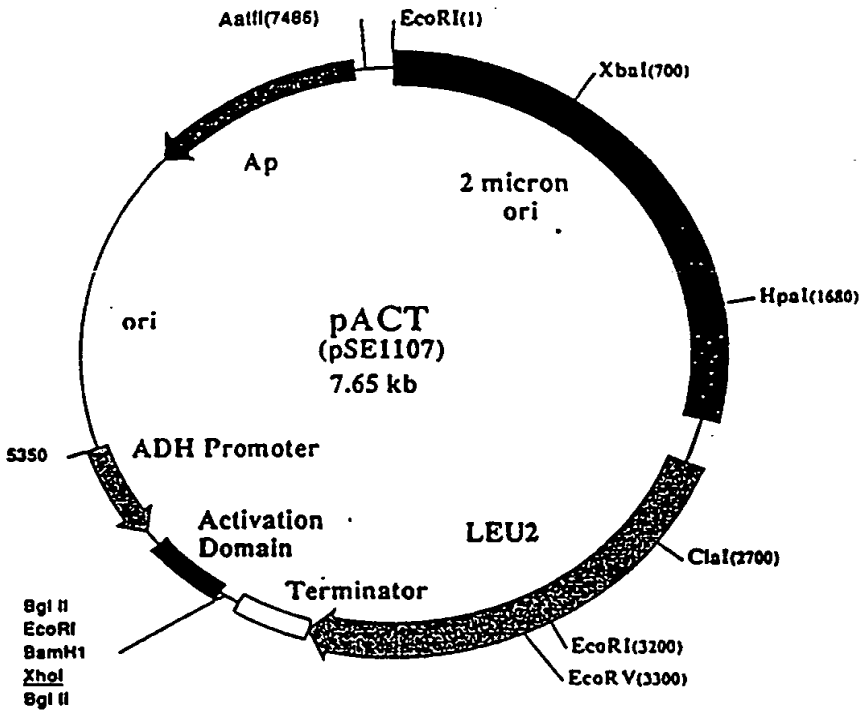
ori
XbaI(1000)
pAS1
7.1 kb
ori
TRP1
XbaI(1900)
EcoRV(2100)
Terminator
HA
NLS
GAL4
(1-147)
P ADH
f1+
ori
SalI (4422)
BamHI
SmaI
SfiI
NcoI
NdeI(4395)
EcoRI(4315)
SacI
BglII
XbaI*
EcoRV(3200)
NaeI(2900)

FIGURE 4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EcoRI(1)
XbaI(700)
Ap
2 micron
ori
HpaI(1680)
ori
pACT
(pSE1107)
7.65 kb
5350
ADH Promoter
Activation
Domain
LEU2
ClaI(2700)
Terminator
Bgl II
EcoRI
BamH1
XhoI
Bgl II
EcoRI(3200)
EcoR V(3300)

FIGURE 5

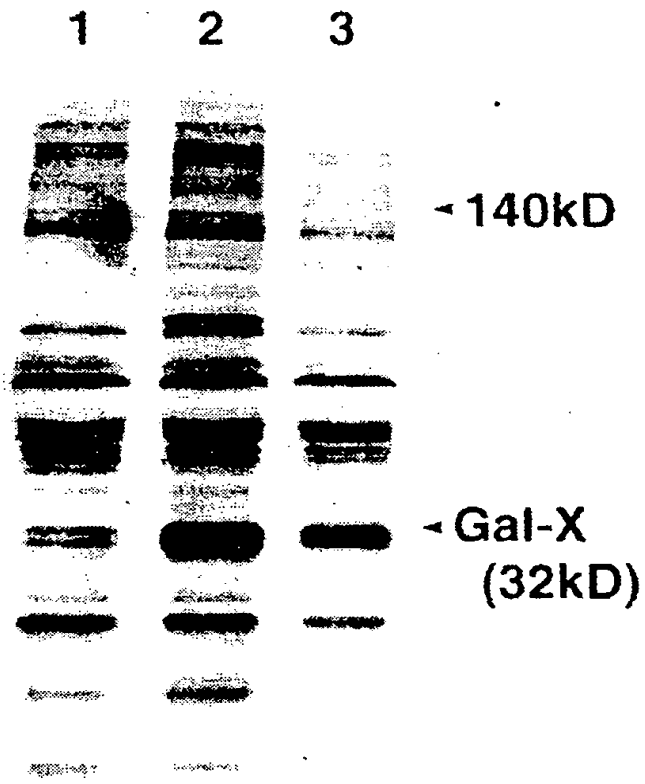

Figure 6

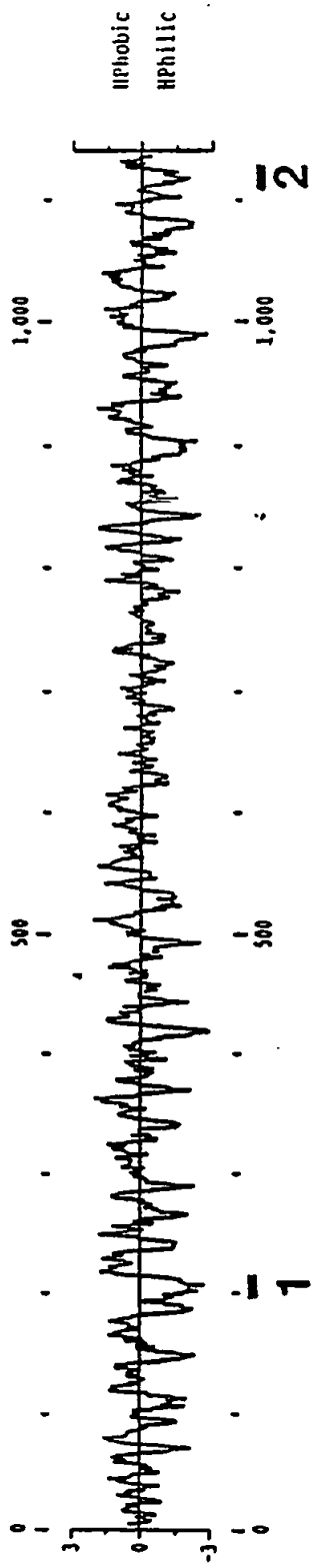

FIGURE 7

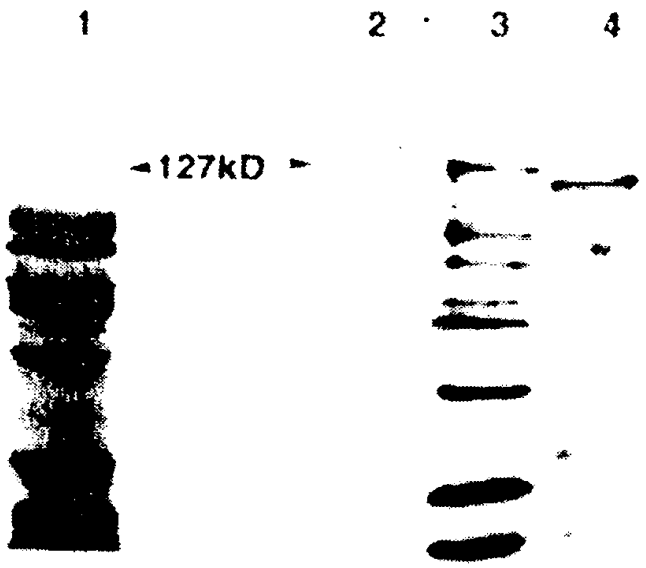

Figure 8

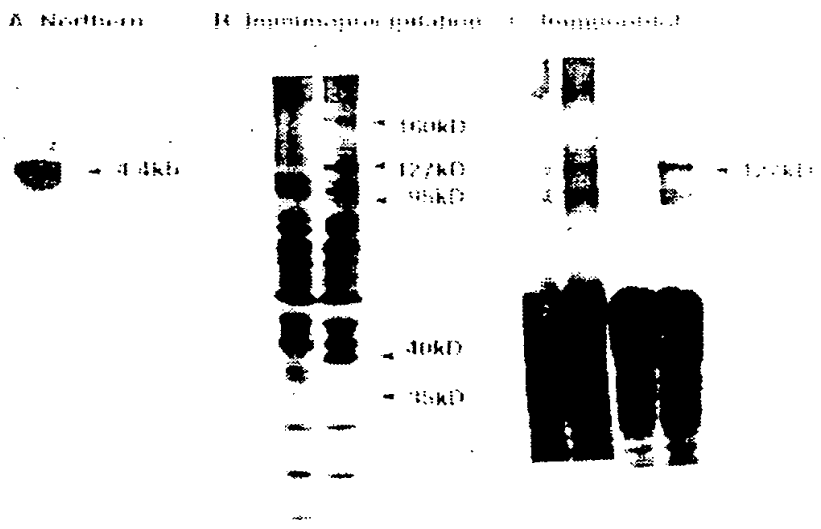
A Northern
B Immunoprecipitation
160kD
127kD
95kD
40kD
35kD

Figure 9

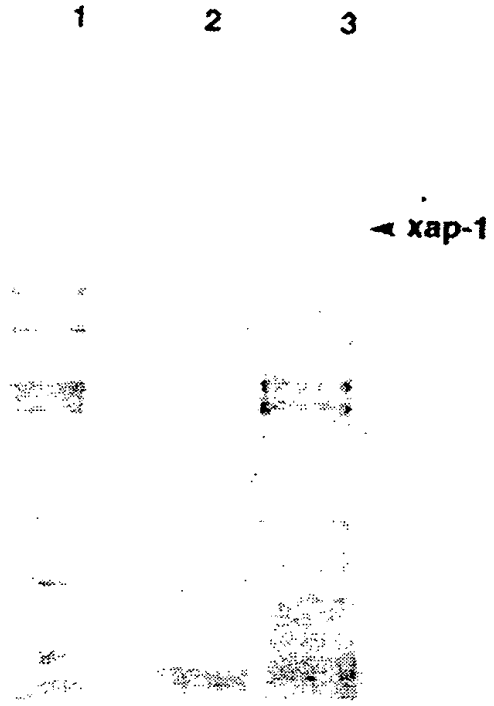

Figure 10

1. clone X ORF into GST fusion vector pGEX-2TK

2. transform E. coli

3. induce the expression of GST-X in bacterial culture

4. harvest cell pellet

5. incubate sonicated cell lysate with glutathione beads

6. wash beads to purify GST-X protein

7. mix with radio-labelled XAP-1 (synthesized in vitro)

8. wash beads and elute the associated proteins

9. SDS-PAGE and autoradiography

FIGURE 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11451

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 184.1; 435/7.1, 7.21; 514/44; 530/358, 387.9; 536/23.5, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, CANCERLIT, DERWENT, JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	Biochemistry, Volume 32, No. 6, issued 16 February 1993, B.J. Hwang et al., "Purification and Characterization of a Human Protein that Binds to Damaged DNA", pages 1657-1666, see pages 1658, column 1; pages 1660-1661, Figures 1 and 2.	8 --- 5, 9-13 --- 1-4, 7
A	B. ALBERTS et al., "MOLECULAR BIOLOGY OF THE CELL", published 1989 by Garland Publishing, Inc. (N.Y.), pages 171-174, 180-192, 1201-1202, see pages 171-174, 180-192, 1201-1202.	1-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
21 NOVEMBER 1994	30 JAN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EVE J. WILSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11451

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	Nucleic Acids Research, Volume 21, Number 17, issued August 1993, M. Takao et al. " A 127-kDa component of a UV-damaged DNA- binding complex, which is defective in some xeroderma pigmentosum group E patients, is homologous to a slime mold protein", pages 4111-4118, see pages 4113 columns 1-2 and Figures 3 and 4.	9, 10 --- 11-13 --- 1-4, 7
Y --- A	Journal of Biological Chemistry, Volume 266, Number 33, issued 25 November 1991, M. Abramic et al., "Purification of an Ultraviolet-inducible, Damage-specific DNA-binding Protein from Primate Cells," pages 22493-22500, see page 22493, column 2; page 22499, column 1.	9-13 --- 1-4, 7
Y --- A	Methods in Enzymology, Volume 70, issued 1980, P.H. Maurer et al., "Proteins and Polypeptides as Antigens", pages 49-70, see pages 49 and 64-70.	9-13 --- 1-4, 7
Y	E. HARLOW et al., "ANTIBODIES A LABORATORY MANUAL", published 1988 by Cold Spring Harbor Laboratory (Cold Spring Harbor, N.Y.), pages 72-76, especially pages 75-76.	10
Y --- A	Progress in Medical Virology, Volume 139, issued 1992, B. Slagle et al., "Hepatitis B Virus and Hepatocellular Carcinoma", pages 167-203, see page 180, second and third full paragraphs.	11-13 --- 1-4, 7
Y --- A	Proceedings of the National Academy of Sciences USA, Volume 87, issued May 1990, G. Chu et al., "Cisplatin-resistant cells express increased levels of a factor that recognizes damaged DNA", pages 3324-3327, see page 3327, column 1.	11-13 --- 1-4, 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11451

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11451

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/70, 38/17, 39/00, 39/395; C07H 21/04; C07K 16/06, 16/18; C12Q 1/00; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 184.1; 435/7.1, 7.21; 514/44; 530/358, 387.9; 536/23.5, 24.31

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1-4, drawn to a method of treating viral disease or cancer by interfering with interaction of the X protein of Hepatitis B virus with the XAP-1 protein of a DNA repair complex.

Group II, claims 5 and 6, drawn to a method for identifying persons at increased risk for developing cancer by detecting alterations in the XAP-1 gene.

Group III, claims 7-8, drawn to a nucleic acid sequence encoding the XAP-1 protein and the amino acid sequence of the XAP-1 protein.

Group IV, claims 9-13, drawn to an antibody which binds to XAP-1 protein and methods using this antibody to detect hepatitis B virus infection, monitor treatment, or determine the stage of liver cancer secondary to hepatitis B virus infection.

The inventions as claimed lack unity under PCT Rule 13 since they are directed to different inventions which are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single general inventive concept. The method of Group II does not recite the XAP-1 protein, nucleic acid, or antibody of Groups I, III, or IV and does not recite the method steps of Group I. The method of Group I does not recite the use of the XAP-1 protein or DNA of Group III in treatment, and does not recite the antibody of Group IV. While the XAP-1 protein is recited in the method of Group I, XAP-1 does not appear to be required as a compound to be added in this method; rather Group I describes interference of the interaction of XAP-1 with X protein in a treatment method. Groups III and IV are not so linked by a special technical feature since Group III is drawn to XAP-1 amino acid and nucleic acid sequences and Group IV is drawn to antibodies and methods using these antibodies.